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Review Article

Insight into the Glucose Metabolism of Immune Cells in Sepsis

Xu Liu, Shui-Jing Wu, and Xiang-Ming Fang

ABSTRACT

Aim of review: To provide a new direction for clarifying the pathogenesis of sepsis and seeking efficient prevention and treatment for sepsis.

Methods: The articles and literature regarding metabolic change of immune cells and/or its role in sepsis published in the last five years were retrieved from PubMed and Web of Science. A basic search was performed using keywords of sepsis, immunity, immune cells, metabolism, etc. In addition, some classical researches and reviews were also referenced.

Recent findings: The cellular metabolism dictates the fate and function of the immune cells, which plays a key role in the occurrence and development of sepsis. Since the immune cells are activated, the cellular metabolism switches from oxidative phosphorylation to aerobic glycolysis (the Warburg effect). The Warburg effect of monocytes/macrophages is the metabolic basis not only for stimulating the inflammatory response but also for trained immunity. In addition, the Warburg effect also contributes to the maturation of dendritic cells and the activation and differentiation of T cells. Studies have shown that the immune cells from septic patients had an impaired ability to stimulate the Warburg effect.

Summary: The immune state of the host is closely related to the metabolism of immune cells. Since cellular metabolism is amenable to pharmacological modulation, further elucidation of this mechanism may provide novel treatment strategies for sepsis. Whether regulating the Warburg effect could help patients survive from sepsis that needs to be further researched.

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Sepsis is defined as life- threatening organ dysfunction caused by an aberrant or dysregulated host response to the invading pathogen (1). There are almost 20 million sepsis cases worldwide each year (2, 3). Although the global outcome of sepsis during the early phase has been significantly improved due to the implementation and promotion of the sepsis bundles including antibiotic therapy, fluid resuscitation and organ function support, the overall mortality remains high (2, 3). Furthermore, the population aging and the increasing incidence of comorbidity (e.g., diabetes) make the pathogenesis of sepsis more complicated, the imbalance between pro- inflammatory and anti- inflammatory response become difficult to interpret the core mechanism in the pathophysiology of this intractable syndrome (Figure 1). Therefore, further clarifying the pathogenesis of sepsis and seeking effective early warning and prevention strategies, which are still major scientific issues in the perioperative medicine and critical care medicine.

The Progress in Understanding of Sepsis and Related Researches

In the 1990s, sepsis was proposed to be

an uncontrolled inflammatory response, "cytokine storm" induced tissue and organ injury even death by changing the regional blood flow, endothelial function, and energy intake and metabolism of parenchymal cells (4-6). However, the following series of multicenter clinical trials to block the overwhelming release of pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1, did not show any benefit on the poor outcome of sepsis (7, 8). Those clinical trials indicate that the role of pro-inflammatory response in patients with sepsis may be unduly exaggerated (9).

In patients with sepsis, the early prognosis is significantly improved by antibiotic therapy as soon as possible, the maintenance of effective hemodynamic stability, and so on (10, 11). However, most surviving patients show a number of characteristics of immunosuppression including apoptotic depletion of immune effector cells and increased expression of negative costimulatory molecules (12, 13). With the shift of dysregulation of inflammatory response to the complicated immune suppression that plays the pivotal role in the occurrence and development of sepsis (11, 14), the prevention and treatment of sepsis-induced immunosuppression has already become a hot spot. Clinical trials aimed to reconstruct the immune response including IL-7 (promotes the proliferation and maturation of CD4+ and CD8+ T lymphocytes, NCT02640807) and anti-programmed cell death ligand 1 (NCT02576457) might be the hope. Despite the results of these trials are still worth pursuing, the results of granulocyte macrophage colony stimulation factor (GM-CSF) and thymosin alpha 1 (TA1) were not optimistic (15, 16). Although GM-CSF could restore markers of monocytic immunocompetence in patients with sepsis, it did not significantly reduce the mortality of septic patients (15), and TA1 could reduce 28-day all-cause mortality of septic patients, but the mortality was still as high as 26% in TA1 group (16). So far, there is still no effective drug for the treatment of sepsis.

The Metabolic Change of Immune Cells in Sepsis

During recent years, a growing number of studies have shown the importance of metabolic signals in immunity, the cellular metabolism dictates the fate and function of the immune cells (17-20). To seek an effective treatment for sepsis, it will be helpful to elucidate metabolic process in the immune cells and analyze their metabolic changes in sepsis (20). It has been found that there is a significant change in the expression of the genes involved in cellular metabolism of glucose in the leukocytes from patients with sepsis via whole genome microarray analysis (19).

Warburg effect, as a mechanism by which the cancer cells rely on glycolysis to power proliferation and biosynthesis even in the presence of oxygen, was first described by German scholar Otto Warburg in the 1920s (20). Following pathogen invasion, the innate immune cells and T cells are activated and generate energy and essential components used in biosynthesis by switching metabolism from oxidative phosphorylation to aerobic glycolysis which is similar to the Warburg effect of tumor cells (Figure 2) (18, 21). Lipopolysaccharide (LPS) stimulation leads to an increase in the glycolytic process, while a decrease in mitochondria-dependent pyruvate oxidation and glutamine consumption, and fatty acid β-oxidation in monocytes/macrophages (22, 23). Furthermore, inhibition of mTOR or deletion of HIF-1α dampens the metabolic change induced by danger signals, which indicates that this metabolic change of immune cells is also mainly regulated by protein kinase B (Akt)-mammalian target of rapamycin (mTOR)-hypoxia inducible factor (HIF)-1α pathway (18, 22-24) (Figure 3). Considering activated immune cells and tumor cells share similar signaling pathway of the metabolic switch, it is reasonable to infer that we can learn from the experiences of the strategies for the prevention and treatment of cancer.

The Warburg Effect and Sepsis

Macrophages are terminally differentiated cells and classified into two major populations: "classic" M1 phenotype (promotes the removal of pathogen by producing pro-inflammatory cytokines and reactive oxygen species) and "alternative" M2 phenotype (inhibits the inflammatory response and promotes tissue repair) (20, 25, 26). The polarization of macrophages to M1 phenotype depends on the Warburg effect (25-27). In contrast, M2-like macrophages have low

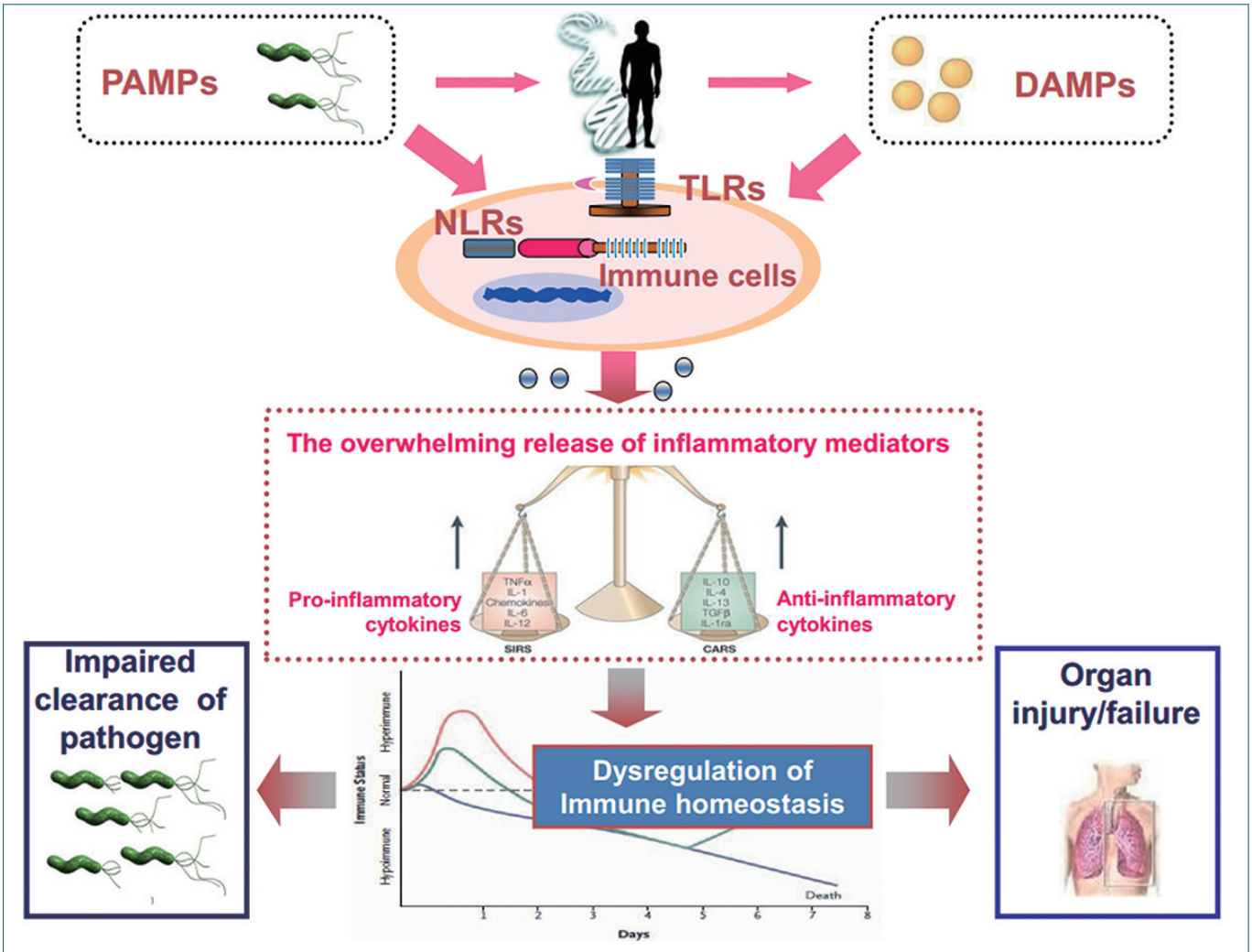

Figure 1. The Immune-Inflammatory Pathogenesis of Sepsis.
PAMPs and DAMPs are recognized by the pattern recognition receptors such as TLRs and NLRs and then induce the overwhelming inflammatory response. The imbalance between pro-inflammatory and anti-inflammatory response leads to the dysregulation of immune homeostasis, which results in the impaired clearance of pathogen and organ injury/failure. DAMPs, damage-associated molecular patterns; NLRs, NOD-like receptors; PAMPs, pathogen-associated molecular patterns; TLRs, Toll-like receptors.

glycolysis rates and high rates of fatty acid β-oxidation and oxidative phosphorylation (25). Inhibition of glycolysis dampens macrophage proinflammatory responses (22).

In addition, the Warburg effect of monocytes/macrophages is also the metabolic basis for trained immunity (trained immunity refers to the memory characteristics of the innate immune system, which can induce nonspecific protection against both infections and malignancies) (20, 23). β-glucan is a component of the Candida albicans cell wall, which could induce trained immunity (23). Cheng SC et al. found that monocytes trained with β-glucan induced epigenetic reprogramming at the level of histone H3 methylation and an increased aerobic glycolysis, and pretreatment with β-glucan improved the survival rate from 40% to 90% in wild-type septic mice (23). Furthermore, metformin (an inhibitor of mTOR signaling) could reverse the trained immunity and significantly increase mortality in mice received the sublethal Candida albicans through inhibiting the Warburg effect (23).

The blood monocytes from septic patients is refractory to endotoxin (e.g., LPS) challenge, the ability to release proinflammatory cytokines is diminished, which has been described as "endotoxin tolerance" (10, 28). Cheng SC et al.

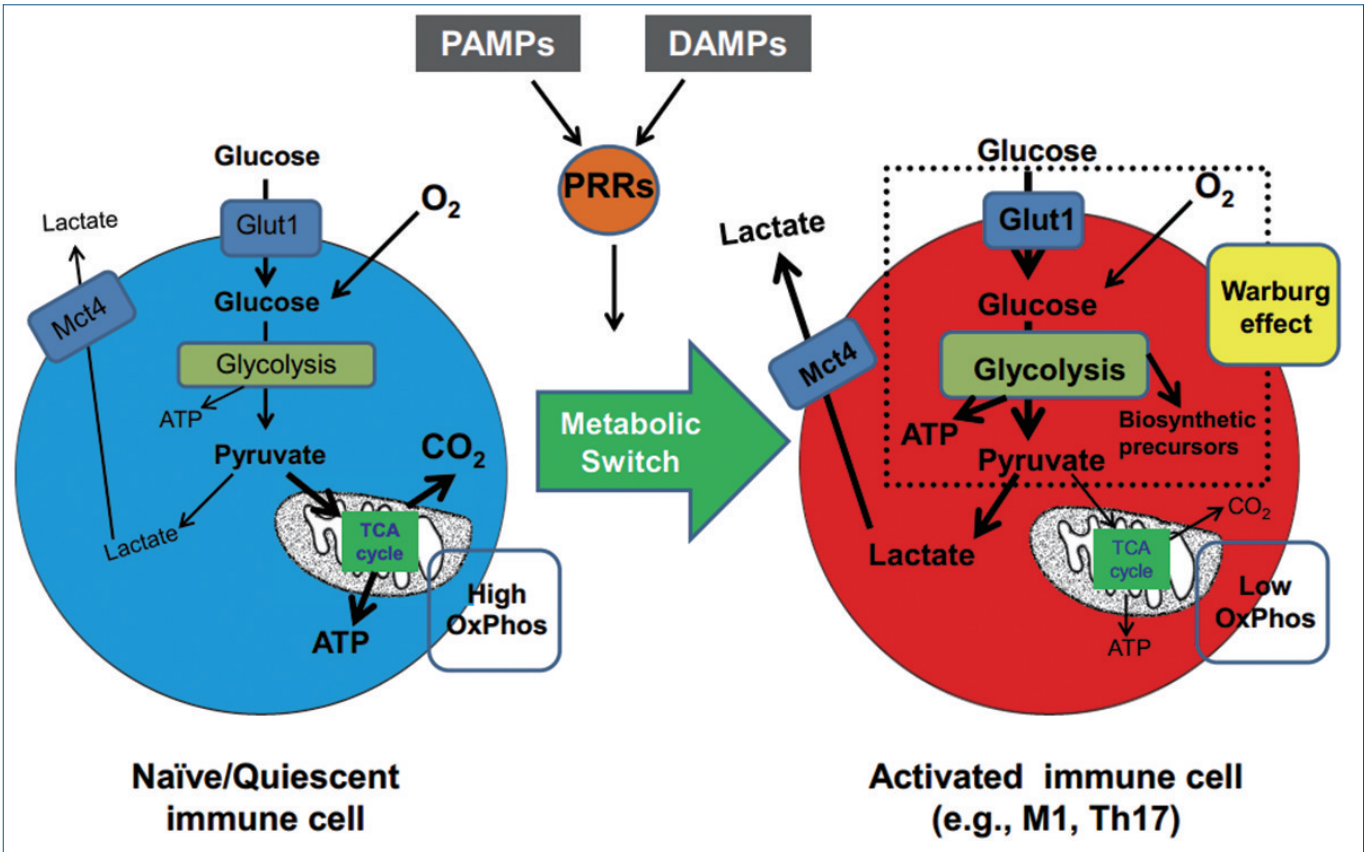

Figure 2. The Metabolism of aActivated Immune cell switches from oxidative phosphorylation to aerobic glycolysis (the Warburg effect).

The left panel shows normal aerobic respiration of naïve/quiescent immune cell with high OxPhos. The immune cells take up small amounts of glucose which is for the most part metabolized to CO_2 and efficiently generated ATP via OxPhos. The right panel shows the metabolism switches to high rates of glycolysis in the activated immune cells. The activated immune cells have an increased expression of Glut1, an increase in glucose uptake and a decrease in the generation of ATP via OxPhos. Glucose is preferentially fermented into lactate even in the presence of oxygen during the activation of immune cells. This metabolic switch increases the biosynthetic capacity of the immune cells and is helpful for rapid generation of ATP. ATP, adenosine triphosphate; DAMPs, damage-associated molecular patterns; Glut1, glucose transporter 1; Mct4, monocarboxylate transporter 4; OxPhos, oxidative phosphorylation; PAMPs, pathogen-associated molecular patterns; TCA, tricarboxylic acid.

found that human peripheral blood mononuclear cells characterizing "endotoxin tolerance" presented a decrease in lactate production and an increase in the production of nicotinamide adenine dinucleotide (NAD^+) after stimulation with LPS, which indicated that a diminished ability of the cells to stimulate the Warburg effect (19). In addition, the monocytes from patients recovered from sepsis had the ability to stimulate the Warburg effect similar to the monocytes from healthy control subjects (19). A recent clinical study showed that ICU-acquired infection was associated with a reduced expression of genes involved in cellular glucose metabolism in the blood leukocytes of septic patients, which might also suggest that there was an impaired ability of leukocytes to stimulate Warburg effect in these patients (29). Therefore, the recovery of the ability of immune cells to stimulate Warburg effect may be beneficial for the prevention and treatment of sepsis and secondary infection in sepsis. Another study showed that the use of 2-deoxy-d-glucose (2-DG) concentration-dependently inhibited the Warburg effect and the release of early (e.g., IL-1β) and late (e.g., high-mobility group box 1 protein, HMGB1) mediators in LPS-stimulated macrophages, and significantly improved 5-day survival rate in rodent models of endotoxic shock and sepsis (30).

However, as previously mentioned, most sep-

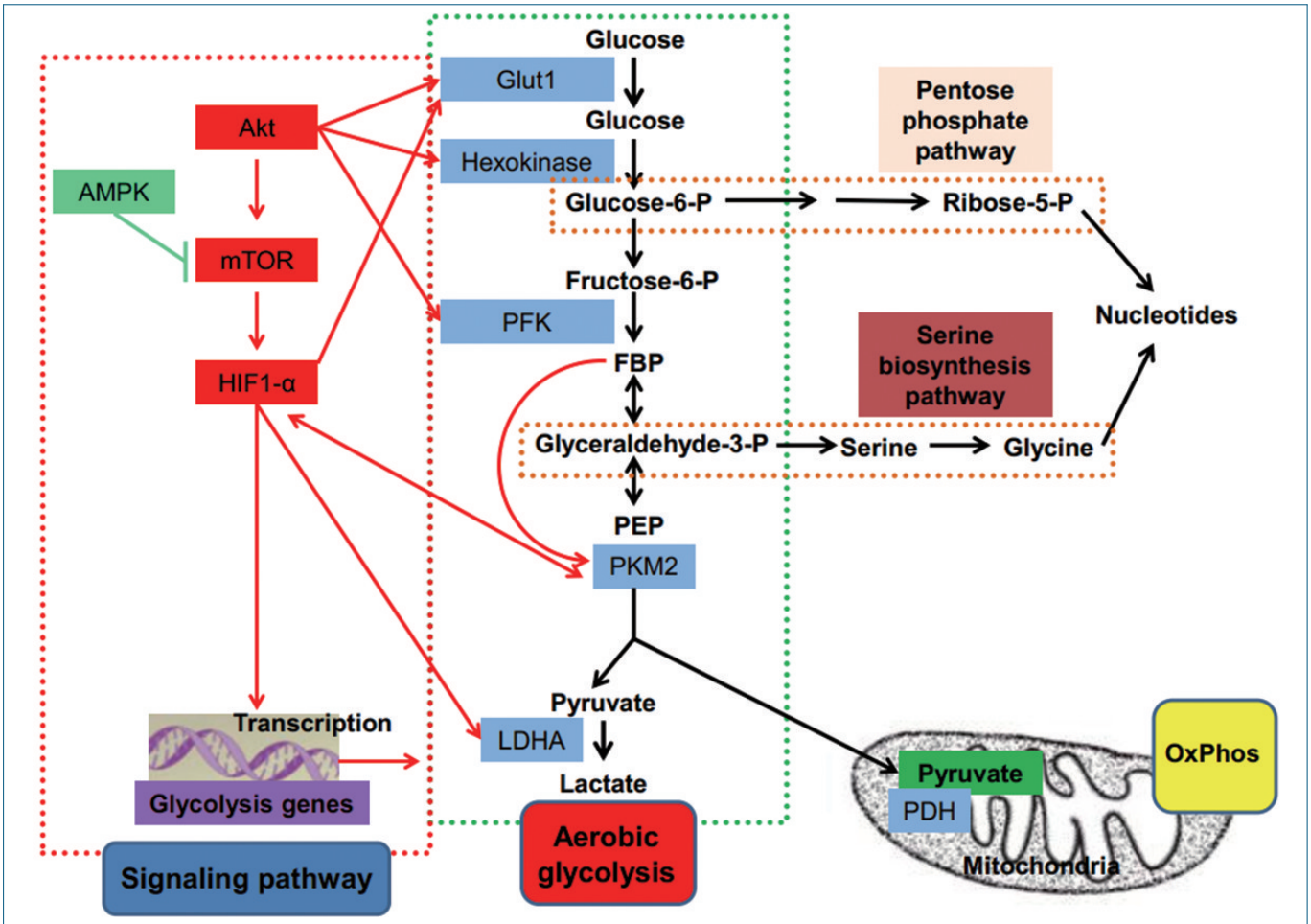

Figure 3. The Metabolic Pathways of Glucose in Activated Immune Cells are Directly Regulated by Signaling Pathways. The left red box shows the signaling pathway which regulates the aerobic glycolysis in activated immune cells. The right green box shows the pathway of cellular glucose fermentation. The serine/threonine kinase Akt stimulates aerobic glycolysis by directly up-regulating glycolytic enzymes or activating mTOR. mTOR facilitates the glycolytic phenotype by enhancing HIF-1α activity. In addition, mTOR is negatively affected by the activation of AMPK. HIF-1α induces the transcription of genes encoding enzymes necessary for aerobic glycolysis, and also directly regulates Glut1 and LDHA, then promotes the aerobic glycolysis in immune cells. HIF-1α can interact with PKM2. In addition, FBP also activates PKM2. The enzymes controlling critical steps in the pathways of cellular glucose metabolism are shown in blue, which might be candidates as novel therapeutic targets in sepsis. Akt, protein kinase B; AMPK, adenosine monophosphate – activated protein kinase; FBP, fructose-1,6-bisphosphatase; Glut1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1α; LDHA, lactate dehydrogenase A; mTOR, mammalian target of rapamycin; OxPhos, oxidative phosphorylation; P, phosphate; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PFK, phosphofructo-kinase; PKM2, pyruvate kinase M2.

tic patients could survive from the early stage of sepsis characterizing "cytokine storm", the main cause of death in sepsis may be immunosuppression because many studies showed that the septic patients could not eradicate the pathogens and tended to develop secondary hospital- acquired infections (9). The abnormal Warburg effect is one of the main features of metabolic defects in leukocytes and an important pathological mechanism of immunoparalysis in sepsis (19). Therefore, regulating the Warburg effect may be a new direction for exploring the effective treatment of sepsis according to the metabolic response of the patients to infection. Interferon (IFN) -γ is the only member of the type II IFN family and has a key role in the immune defense against viruses, bacteria and protozoal infections (10). The laboratory study revealed that IFN-γ could restore the ability of endotoxin tolerance monocytes to stimulate the Warburg effect through the mTOR pathway (19). Several case reports also showed that IFN- γ could reverse monocyte dysfunction and improve the outcome of septic patients with a reduced ex-

pression of human leukocyte antigen complex (HLA- DR) (31, 32). However, further studies are still needed to confirm the effect of IFN-γ on the Warburg effect of monocytes and the beneficial role in the patients with sepsis.

In addition, we should also pay attention to the role of the Warburg effect in dendritic cells, T cells and other immune cells. The Warburg effect contributes to the maturation of dendritic cells in response to Toll like receptor (TLR) 4, TLR2, and TLR9 ligands (30). Administration of 2-DG strongly blocks the activation of dendritic cells and reduces the ability of dendritic cells to present antigens to T cells by antagonizing mTOR signaling (17). A metabolic hallmark of T cell activation is the Warburg effect, which is important for promoting T cell effector function such as secreting IFN-γ (33, 34). When T cells using aerobic glycolysis were forced to oxidative phosphorylation, their ability to produce IFN-γ was markedly compromised (33). Furthermore, T cell differentiation is also controlled by the cellular metabolism of glucose, such as anti-inflammatory Treg cells switch to low levels of glycolysis and preferentially use oxidative metabolism (35). Inhibition of the Warburg effect promotes the differentiation of Treg cells, but reduces the differentiation of pro-inflammatory Th17 cells (20, 30, 35, 36).

In a word, the Warburg effect affects the activation, differentiation and memory formation of the immune cells. Focus on the Warburg effect of immune cells in sepsis would help us to improve the current treatment strategies even discover new methods for the prevention and treatment of sepsis.

Summary and Perspective

Following the pathophsiology of sepsis was uncovering, the prognosis of septic patients at the early stage with a hyperinflammation status has been significantly improved. However, the mortality at late stage of sepsis is still high (10). The pathophysiological mechanisms become much complicated due to immunosuppression and/or secondary infection at the late stage (several days to a few weeks after being diagnosed as sepsis) in septic patients. It is still uncertain whether the treatment strategies for enhancing the host immunity could effectively reduce mortality of sepsis. More and more evidences reveal that the metabolism of the immune cells is not only regulated by the oxygen and nutrients, but also affected by danger signals. Although it has been recognized that metabolic changes of immune cells may play an important role in the pathogenesis of sepsis, the relevant research evidence is still inadequate. Akt-mTOR-HIF-1α signaling pathway and the enzymes related to glycolysis such as hexokinase are potential targets for regulating the Warburg effect (Figure 3). The role of these targets in immunometabolism and the mechanism underlying the Warburg effect in host response to infection and organ dysfunction need to be further investigated. Since the Warburg effect is amenable to pharmacological modulation, further elucidation of this mechanism might help us to improve the current treatment strategies even discover new methods for the prevention and treatment of sepsis.

The activation of immune cells elicit the Warburg effect similar to tumor cells, so we can draw lessons from the ideas, evidences and strategies for individual prevention and treatment of cancer. It has a promising future that individual intervention for regulating the Warburg effect according to the specific host/pathogen genomic patterns and the state of cellular metabolism in immune cells. For example, restoring the impaired Warburg effect in patients with sepsis may reduce secondary infection, and triggering the Warburg effect to induce trained immunity in elderly people with immunosenescence may be useful for the prevention and treatment of sepsis. In addition, considering that the inherent complexity and interaction of immunity and metabolism, it is recommended that the immune function and cellular metabolism should be closely monitored when the clinical trials are conducted so that appropriate treatment strategy could be used to improve the outcomes of sepsis. Monitoring and regulating immune cell metabolism, especially the Warburg effect, should be the key and a new breakthrough point to prevent and treat sepsis.

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The authors have no potential conflict of interest for this review to declare.

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